

WHAT IS CLAIMED:

1 1. An expression plasmid comprising an RNA polymerase I (pol I)
2 promoter and pol I terminator sequences, which are inserted between an RNA polymerase II
3 (pol II) promoter and a polyadenylation signal.

Sub B1
1 2. The expression plasmid of claim 1 wherein the pol I promoter is
2 proximal to the polyadenylation signal and the pol I terminator sequence is proximal to the pol
3 II promoter.

1 3. The expression plasmid of claim 1 wherein the pol I promoter is
2 proximal to the pol II promoter and the pol I terminator sequence is proximal to the
3 polyadenylation signal.

1 4. The expression plasmid of claim 1 wherein the plasmid corresponds to
2 a plasmid having a map selected from the group consisting of pHW2000, pHW11 and
3 pHW12.

1 5. The expression plasmid of claim 1, further comprising a negative strand
2 RNA virus viral gene segment inserted between the pol I promoter and the termination signal.

1 6. The expression plasmid of claim 5, wherein the negative strand RNA
2 virus is a member of the *Orthomyxoviridae* virus family.

1 7. The expression plasmid of claim 6, wherein the virus is an influenza A
2 virus.

1 *Sub 1* 8. The expression plasmid of claim 7, wherein the viral gene segment
2 encodes a gene selected from the group consisting of a viral polymerase complex protein, M
3 protein, and NS protein; wherein the genes are derived from a strain well adapted to grow in
4 cell culture or from an attenuated strain, or both.

1 9. The expression plasmid of claim 6, wherein the virus is an influenza B
2 virus.

1 10. The expression plasmid of claim 8 wherein the plasmid has a map
2 selected from the group consisting of pHW241-PB2, pHW242-PB1, pHW243-PA, pHW245-
3 NP, pHW247-M, and pHW248-NS.

1 11. The expression plasmid of claim 8 wherein the plasmid has a map
2 selected from the group consisting of pHW181-PB2, pHW182-PB1, pHW183-PA, pHW185-
3 NP, pHW187-M, and pHW188-NS.

1 *Sub B3* 12. The expression plasmid of claim 7, wherein the viral gene segment
2 encodes a gene selected from the group consisting of an influenza hemagglutinin (HA) gene
3 and a neuraminidase (NA) gene.

1 13. The expression plasmid of claim 12, wherein the influenza gene is from
2 a pathogenic influenza virus strain.

1 *Sub B4* 14. The expression plasmid of claim 12, wherein the plasmid has a map
2 selected from the group consisting of pHW244-HA, pHW246-NA, pHW184-HA, and
3 pHW186-NA.

1 15. A minimum plasmid-based system for the generation of infectious
2 negative strand RNA viruses from cloned viral cDNA comprising a set of plasmids wherein
3 each plasmid comprises one autonomous viral genomic segment, and wherein the viral cDNA
4 corresponding to the autonomous viral genomic segment is inserted between an RNA
5 polymerase I (pol I) promoter and terminator sequences, thereby resulting in expression of
6 vRNA, which are in turn inserted between a RNA polymerase II (pol II) promoter and a
7 polyadenylation signal, thereby resulting in expression of viral mRNA.

1 16. The minimum plasmid-based system of claim 15 wherein the pol I
2 promoter is proximal to the polyadenylation signal and the pol I terminator sequence is
3 proximal to the pol II promoter.

1 17. The minimum plasmid-based system of claim 15 wherein the pol I
2 promoter is proximal to the pol II promoter and the pol I terminator sequence is proximal to
3 the polyadenylation signal.

1 18. The plasmid-based system of claim 15, wherein the negative strand
2 RNA virus is a member of the *Orthomyxoviridae* virus family.

1 19. The plasmid-based system of claim 18, wherein the virus is an
2 influenza A virus.

1 20. The plasmid-based system of claim 18, wherein the virus is an
2 influenza B virus.

1 21. The plasmid-based system of claim 19, wherein the viral gene segment
2 encodes a protein selected from the group consisting of a viral polymerase complex protein,
3 an M protein and an NS protein; wherein said genes are from a strain well adapted to grow in
4 cell culture or from an attenuated strain, or both.

1 22. The plasmid-based system of claim 19, wherein the viral genomic
2 segments comprise genes which encode a protein selected from the group consisting of

- 1 hemagglutinin and neuraminidase, or both; wherein said genes are from a pathogenic
2 influenza virus.

1 23. The plasmid-based system of claim 19 wherein said system comprises
2 one or more plasmids having a map selected from the group consisting of pHW241-PB2,
3 pHW242-PB1, pHW243 -PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and
4 pHW248-NS.

1 24. The plasmid-based system of claim 19, wherein said system comprises
2 one or more plasmids having a map selected from the group consisting of pHW181-PB2,
3 pHW182-PB1, pHW183 -PA, pHW184-HA, pHW185-NP, pHW186-NA, pHW187-M, and
4 pHW188-NS.

1 25. A host cell comprising the plasmid-based system of claim 15.

1 26. A host cell comprising the plasmid-based system of claim 18.

1 27. A host cell comprising the plasmid-based system of claim 19.

1 28. A host cell comprising the plasmid-based system of claim 22.

1 29. A method for producing a negative strand RNA virus virion, which
2 method comprises culturing the host cell of claim 25 under conditions that permit production
3 of viral proteins and vRNA or cRNA.

1 30. A method for producing an *Orthomyxoviridae* virion, which method
2 comprises culturing the host cell of claim 26 under conditions that permit production of viral
3 proteins and vRNA or cRNA.

1 31. A method for producing an influenza virion, which method comprises
2 culturing the host cell of claim 27 under conditions that permit production of viral proteins
3 and vRNA or cRNA.

1 32. A method for producing a pathogenic influenza virion, which method
2 comprises culturing the host cell of claim 28 under conditions that permit production of viral
3 proteins and vRNA or cRNA.

1 33. A method for preparing a negative strand RNA virus-specific vaccine,
2 which method comprises purifying a virion produced by the method of claim 29.

1 34. The method according to claim 33, which further comprises
2 inactivating the virion.

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1 35. The method according to claim 33, wherein the negative strand RNA
2 virus is an attenuated virus.

1 36. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises administering a protective dose of a vaccine of claim 33 to
3 the subject.

1 37. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises injecting a protective dose of a vaccine of claim 33
3 intramuscularly in the subject.

1 38. A method for vaccinating a subject against a negative strand RNA virus
2 infection, which method comprises administering a vaccine of claim 33 intranasally to the
3 subject.

1 39. A method for generating an attenuated negative strand RNA virus,
2 which method comprises:
3 (a) mutating one or more viral genes in the plasmid-based system of claim
4 15; and
5 (b) determining whether infectious RNA viruses produced by the system
6 are attenuated.

1 41. A composition comprising a negative strand RNA virus virion
2 produced by the method of claim 29.

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